GEORGIA INSTITUTE OF TECHNOLOGY — OFFICE OF CONTRACT ADMINISTRATION

PROJECT ADMINISTRATION DATA SHEET

[x] ORIGINAL [ ] REVISION NO. _____

Project No. G-33-692 GTRI/~~GIT~~ DATE 4 / 25 / 84

Project Director: Kent Barefield School/~~Dept~~ Chemistry

Sponsor: David Labs, Inc.; Atlanta, GA

Type Agreement: Research Project Agreement dated 4/20/84

Award Period: From 4/20/84 To ~~10/19/84~~ 12-31-84 (Performance) 10/19/84 (Reports)

Sponsor Amount:	This Change	Total to Date
Estimated: $		$ 6,000
Funded: $		$ 6,000

Cost Sharing Amount: $ _____ Cost Sharing No: _____

Title: Experimental Evaluation of Methods for Preparation of Cholesterol Oleate

ADMINISTRATIVE DATA OCA Contact John W. Burdette x4820

1) Sponsor Technical Contact:

2) Sponsor Admin/Contractual Matters:

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(404) 955-7486

Defense Priority Rating: n/a Military Security Classification: n/a

(or) Company/Industrial Proprietary: n/a

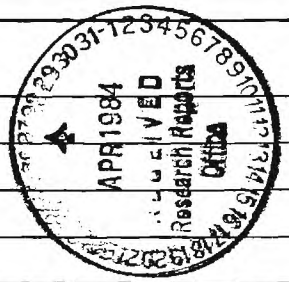
RESTRICTIONS

See Attached ----- Supplemental Information Sheet for Additional Requirements.

Travel: Foreign travel must have prior approval – Contact OCA in each case. Domestic travel requires sponsor approval where total will exceed greater of $500 or 125% of approved proposal budget category.

Equipment: Title vests with none proposed.

COMMENTS:



Sponsor I.D. #02.253.000.84.001

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FORM OCA 4:383

GEORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION/CLOSEOUT SHEET

Date 5/17/85

Project No. G-33-692 School/~~Lab~~ Chemistry

Includes Subproject No.(s)

Project Director(s) Dr. Kent Barefield GTRI / ~~GIT~~

Sponsor David Labs Inc.

Title Experimental Evaluation of methods for Preparation of Cholesterol Oleate.

Effective Completion Date: 12/31/84 (Performance) 12/31/84 (Reports)

Grant/Contract Closeout Actions Remaining:

- [ ] None
- [XX] Final Invoice or Final Fiscal Report
- [ ] Closing Documents
- [ ] Final Report of Inventions
- [ ] Govt. Property Inventory & Related Certificate
- [ ] Classified Material Certificate
- [XX] Other Copy of final report to OCA reports (sponsor has-received the final report and requested project be terminated by OCA)

Continues Project No. Continued by Project No.

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EXPERIMENTAL EVALUATION OF METHODS OF SYNTHESIS OF CHOLESTERYL OLEATE

Report prepared for

David Labs, Inc.

by

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Objective.

The objective of this work was to establish a reliable and cost effective method for the synthesis of cholesteryl oleate with properties suitable for use in liquid crystal admixtures.

Introduction.

The following methods have been reported for the synthesis of fatty acid esters of cholesterol (R = cholesteryl group, $C_{27}H_{45}$; R' = alkyl group of acid):

$$ROH + R'CO_2H \xrightarrow{200°C} R'CO_2R + H_2O \qquad (1)^1$$

$$ROH + R'CO_2H \xrightarrow{H^+,\ C_6H_6} R'CO_2R + H_2O \qquad (2)^2$$

$$ROH + R'CO_2H \xrightarrow[\text{agent}]{\text{coupling}} R'CO_2R \qquad (3)^3$$

$$2\ R'CO_2H \xrightarrow[\text{agent}]{\text{coupling}} (RCO)_2O \qquad (4)^4$$

$$ROH + (R'CO)_2O \longrightarrow R'CO_2R + RCO_2H$$

$$RO_2CCH_3 + R'CO_2CH_3 \xrightarrow{NaOR''} R'CO_2R + CH_3CO_2CH_3 \qquad (5)^5$$

$$R'CO_2H + SOCl_2 \longrightarrow R'COCl + SO_2 + HCl \qquad (6)^{6,7}$$

$$ROH + R'COCl \longrightarrow R'CO_2R + HCl$$

All of the above methods except (2) have been used for the preparation of the oleate ester (R' = $C_{17}H_{33}$). None of the literature preparations of cholesteryl oleate were conducted on more than a 10 mmol scale and most were on a micro or semimicro scale. The greatest interest in this material has apparently been in connection with its physiological role rather than for its liquid crystal properties. Purification has typically required column chromatography. In view of the ultimate goal of preparing large quantities of the ester at low cost, preparative methods (1)-(4) can be ruled out. Methods (1) and (2) are expected to result in extensive decomposition and/or isomerization of the oleic acid, which will result in a product that would be purifiable only by chromatography, if at all. Methods (3) and (4) require reagents that are too expensive for use on a large scale. Both methods (5) and (6) appear to be applicable to large scale syntheses with (6) being potentially the most cost effective at least in terms of the costs of the starting materials.

David Fuller's inquiries concerning the synthesis suggested that the commercial material was prepared via the acid chloride method so that this route was targeted for initial investigation. A large number of attempts to prepare the oleate ester were made before the material was satisfactorily crystallized. However, the procedure given below gave crystalline material in reproducible fashion and should be applicable to any scale. Attempts to apply method 5 (above) according to the literature procedure were not encouraging.

Reactions were not complete in the times designated in the procedure and the reaction mixtures were difficult to stir.

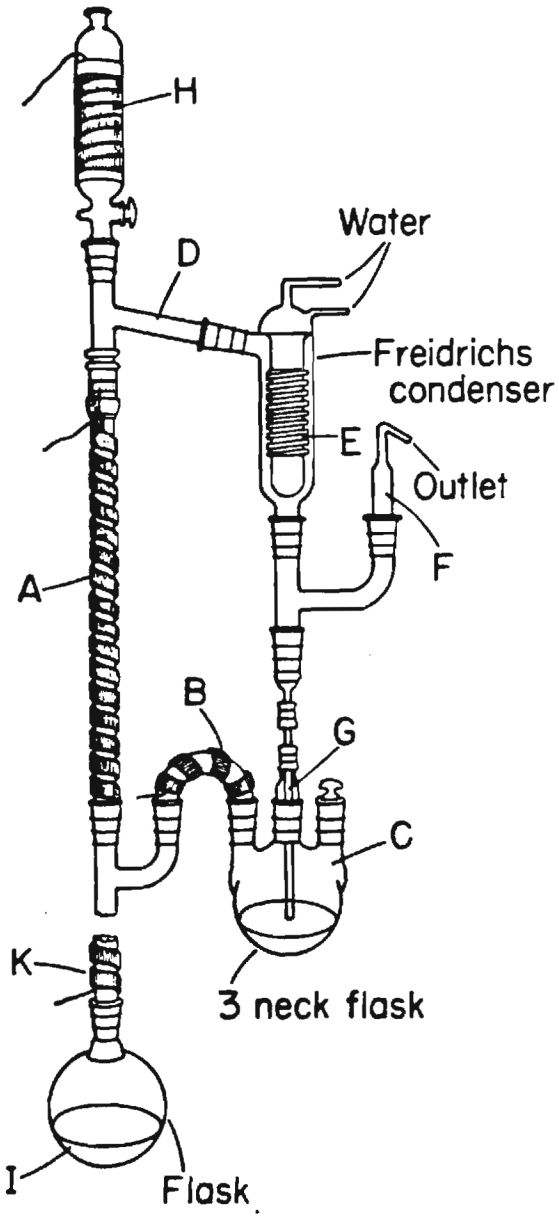
Preparation of Cholesteryl Oleate.

Step 1: Preparation of Oleoyl Chloride.

$$C_{17}H_{33}CO_2H + SOCl_2 \text{ ----------> } C_{17}H_{33}COCl + SO_2 + HCl$$

Two methods were utilized: heating oleic acid with excess thionyl chloride (batch process) until the OH and C=O stretching absorptions of the acid disappeared and use of a counter-current reactor (Figure 1), which limits contact time of the acid chloride to the reaction temperature. The latter method gave less highly colored acid chloride and is therefore recommended since the ease of crystallization of cholesteryl oleate appears to be directly related to the color of the reaction mixture.

Procedure: After flushing the reactor with dry nitrogen, reflux of thionyl chloride in the reactor was established by preheating the column (A) to about 60°C and tube K to about 80°C and then heating the 3-necked flask C with a heating mantle. Dropping funnel H was charged with oleic acid, a fresh receiver I was attached and a slow flow of acid down the column was begun. Flow rates of 10mL/15 min through a 1/2" column resulted in complete conversion of the acid to the chloride (disappearance of the O-H and C=O stretching absorptions of the acid at 3200 cm^{-1} and 1715 cm^{-1}, respectively). No attempt was made to determine a maximum flow rate. Slower flow rates resulted in somewhat more thionyl chloride in the receiver flask but the quality of the product was equally good. A few minutes after all of the acid had drained from the dropping funnel, the heating devices were


H
Water
D
Freidrichs
condenser
E
Outlet
F
A
B
G
C
K
3 neck flask
I
Flask

shut off and the receiver was removed and the excess thionyl chloride was removed on a rotary evaporator at 50-60°C and 25-30 torr. The oleoyl chloride was checked by infrared spectroscopy to determine that all of the thionyl chloride was removed as evidenced by the absence of a S=O stretching absorption at 1236 cm^{-1}. Failure to remove all of the $SOCl_2$ gave an esterification product mixture that did not crystallize. The color of the oleoyl chloride obtained depended on the color of the oleic acid used for the preparation: colorless, gold label acid obtained from Aldrich Chemical Co. gave a light straw yellow chloride; light amber acid obtained from Lancaster Synthesis Ltd (advertised as 97%) gave a fairly dark orange brown chloride. The acid obtained from Aldrich showed no evidence of any trans isomer (C-13 NMR). The acid obtained from Lancaster contained detectable amounts of three unsaturated species amounting to ca. 20% of the total unsaturates. Examination of the oleoyl chloride obtained from Lancaster acid by ^{13}C NMR spectroscopy indicated no change in the composition from that of the acid.

Step 2: Reaction of Oleoyl Chloride with Cholesterol

$$C_{17}H_{33}COCl + C_{27}H_{46}O \text{ -------> } C_{27}H_{46}OC(O)C_{17}H_{33}$$

A weighed quantity of oleoyl chloride (6-16 g, 21-56 mmol) was placed in a nitrogen flushed 3-necked flask fitted with mechanical stirrer and nitrogen inlet equipped with pressure relief bubbler. One equivalent of cholesterol was added and the flask was heated at 60°C with an oil bath. Within 30 min most of the mixture had liquified and stirring was commenced. An alternate procedure was to heat the oleoyl chloride to 60° C and

add the cholesterol in portions. Since HCl is evolved, this method must be used for large scale reactions with HCl evolution controlled by the rate of cholesterol addition. Heating and stirring was continued for 3 h. The reaction mixture was yellow to dark orange-brown at this stage depending upon the color of the oleoyl chloride used for the preparation. Extended reaction times resulted in the appearance of two or three additional components in thin layer chromatograms of the reaction mixtures. After cooling to room temperature, the viscous reaction product was dissolved in 50-100 mL of hexane or medium petroleum ether and was treated with 1-3 g of activated charcoal. Gravity filtration through filter paper at this point gave a much lighter solution; however, it was found that filtration of the solution through a 1-3 cm pad of silica gel on a 60 ml sintered glass funnel gave a nearly colorless filtrate and a product with a higher melting point. After such treatment, the filtrate was placed on a rotary evaporator and the solvent removed at 50-60°C and 25-30 torr. The residue crystallized upon standing at room temperature for 6-15 h. The solid product was broken up with a stirring rod, 75-150 ml of ethanol was added to the flask and the mixture was stirred vigorously at 0°C with a mechanical stirrer to give a finely divided white or slightly yellow product. Melting points ranged from 41-42°C for material prepared with reagent grade cholesterol (Fisher) and Lancaster Synthesis, Ltd oleic acid (samples #1 and #2) to 43-44°C for material prepared with primary standard grade cholesterol (Kodak) and the Aldrich gold label oleic acid (sample #3). Yields were

75-85%. There did not seem to be a good correlation of clearing point with either color or melting point of the material. Clearing points for samples #1, #2 and #3 were 45°C, 45.5°C and 44.5°C, respectively.

Step 3: Purification

Recrystallization of cholesterol oleate was difficult. It is extremely soluble in both hot and cold hexane, benzene, chloroform and methylene chloride. It has very insoluble in cold acetone and ethanol with somewhat higher solubility in these solvents when hot. In principle, recrystallization should be possible by addition of ethanol to a chloroform solution of the ester. This method was attempted using Janssen ester, lot #20681. Although crystallization was ultimately accomplished by addition of ethanol to a solution of the ester at room temperature to near the permanent cloud point and then placing the solution in a refrigerator, the ester usually oiled and the procedure had to be repeated several times before nucleation was achieved.

A more reliable purification could be achieved by either of the following methods. In the first method, which may be more easily applicable to larger quantities, the ester was stirred in boiling ethanol for 10-15 min, the mixture was cooled in an ice bath and the ethanol decanted. This procedure was repeated a second time except that the mixture was stirred vigorously with a mechanical stirrer while it cooled in the ice bath. The nearly white solid was collected by filtration and dried under vacuum. The melting point of portions of sample #3 were not improved but

the clearing point increased from 45.5°C to 48.8°C. In the second method, sufficient ethanol was used to dissolve nearly all of the sample at about 75°C. The mixture was gravity filtered to remove any particulate matter and undissolved oily material. The filtrate was then stirred vigorously while cooling in an ice bath. One gram portions of sample #3 required ca. 40 mL of ethanol but were obtained as pure white materials. The clearing point was increased from 45.5°C to 49.7°C by this procedure. Melting points were determined by the capillary method. Clearing points were determined by visual detection of the loss of color upon very slow heating of the sample in a water bath. This visual technique can probably be improved upon by degassing a sample and sealing it in an evacuated ampoule since the sharpness of the clearing point is frequently obscured by air bubbles in the sample. A sample of Janssen #20681 prepared in this fashion melted to give a perfectly clear fluid.

^{13}C Analysis of Commercial Samples.

^{13}C spectra were obtained on 6 commercial samples of cholesteryl oleate that were supplied by David Fuller. The ^{13}C spectra (obtained in C_DCl_3 vs TMS as internal standard) of these samples were virtually identical. Each contained only a single carbonyl carbon resonance and all except #27733 contained two pairs of olefinic carbon resonances resulting from the oleoyl moiety. The more intense pair of these resonances occurred at 129.7 and 129.9 ppm and the less intense pair at 130.15 and 130.4 ppm. These can be assigned to the (Z)-9-octadecenoic (cis) and (E)-9-octadecenoic (trans) acid derivatives, respectively based

on their relative chemical shifts and also relative intensities. The amount of the (E) or trans isomer present in each sample was estimated by the ratio of the integrals of its olefinic resonances to the total integral of the (Z) and (E) isomers:

Sample #	Percent Trans Isomer
27733	none detectable
019606	18
22463	17
20681	19
Test A	19
Test B	20

All samples except #27733 had a more complex spectrum in the 29 ppm region than observed for pure oleic acid. This is additional evidence for the presence of something other than oleate.

Examination of sample #20681 by thin layer chromatography (silica gel, hexane:benzene 6:4 v/v) also indicated the presence of a minor (R_f = 0.5) and major component (R_f = 0.57). Sample #27733 showed little, if any, minor component by TLC.

All commercial samples except #27733 exhibited low intensity resonances at 32.5 and 32.6 ppm. This is the region in which the t-butyl methyl groups of BHT (see next section) should occur. If one of these resonances is due to BHT then it is not present in detectable amounts in #27733 or in the experimental samples.

Analyses of Experimental Samples

^{13}C spectra of experimental samples #1-3 showed no evidence

for the presence of trans oleate. However, samples #1 and #3, which were prepared from Lancaster acid show a more complex pattern of methylene resonances in the 29 ppm region (due to oleate) than are present in #2 or in #27733. The presence of a small amount of a minor component was also suggested by TLC. This minor component is probably a saturated acid ester. The absence of other unsaturated species indicates that they are removed by the ethanol wash, since they are clearly present in the crude reaction product. Sample #2 showed no resonances for other unsaturated acids and the sharpness of the oleate methylene resonances suggests a high degree of purity.

Mass Spectral Analysis of Commercial Sample 20681.

An attempt was made to determine what solvent was used in the purification of sample #20681 by mass spectrometry. The sample gave no discernable solvent peaks but intense peaks corresponding to 2,6-bis(1,1-dimethylethyl)-4-methylphenol (BHT) were obtained upon slight warming of the sample. The origin of the BHT is unknown. It might be noted that a molecular ion for cholesteryl oleate was readily detected.

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